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from it as shown in the illustration. (Fig. 2.) The board is mounted near the center on an extension shaft which is fitted with two joints, the one at the end to which the board is attached being a ball-and-socket joint and the other an adjustable swivel joint. The shaft is screwed into a metal base which has sufficient weight to hold the board steady when placed in any position.

The procedure for making the injection is as follows: With the board properly placed in a horizontal position, the animal is tied to it securely, abdomen downward, by means of strings. The board is then placed in a vertical position and rotated on its vertical axis slightly so as to bring the dorsal aspect of the right hind leg into view. After clipping the hair from the leg and shaving it, the leg is lifted up slightly by the first or first and second fingers and the vein dilated by suitable compression. (Fig. 6.) The vessel can now usually be seen through the skin. A small incision, usually about one-fourth of an inch long, is made diagonally across the leg from the outer lower to the upper and inner aspect, but a trifle to the left of the vessel. The subcutaneous tissue is then pushed aside with a fine pointed forceps, thereby permitting the vessel to come into view.

The vessel is then entered directly (Fig. 3) or in the same manner as has been described for the rat—that is, by passing the needle of the tuberculin syringe through the fascia and muscles to the left of the vessel and then entering the vessel from the side. The vessel when dilated permits the ready entrance of a No. 23 B. & S. gauge needle. However, the needle usually employed is a No. 26 B. & S. gauge, five-eighths of an inch in length. The needle is always introduced well into the lumen of the vein. If entrance into the vessel is direct, subsequent hemorrhage may be controlled readily by pinching it with a small forceps.

PRELIMINARY NOTE ON A STABLE SILVER VITAMINE COMPOUND OBTAINED FROM BREWER'S YEAST.

By ATHERTON SEIDELL, Technical Assistant, United States Public Health Service.

Since the discovery in 1915¹ that fullers' earth possesses a remarkable adsorptive power for vitamine, the product resulting from this attraction has been used by me as the starting point for all subsequent attempts to isolate a pure, stable, antineuritic compound. The vitamine-fullers' earth combination has, for convenience, been designated as "activated" fullers' earth, and a large quantity of it was prepared and carefully standardized for its antineuritic power by tests on pigeons. Repeated tests on some of the samples showed that no loss of activity occurred during a period of more than five years. Until recently all of the attempts to obtain a pure vitamine

¹ Seidell, Atherton, "A Stable Form of Vitamine, etc.": Public Health Reports, 31, 364-370, Feb. 18, 1916.

from "activated" fullers' earth have yielded products which, although highly antineuritic, were not sufficiently stable or well characterized to warrant further study as to their composition. The work up to now has, therefore, been useful only in showing the procedures by which a well-defined antineuritic can not be obtained.

Briefly, "activated" fullers' earth is prepared as follows: Well-washed and pressed brewer's yeast is allowed to autolyze in a warm place for 48 hours or more. The resulting thick liquid is filtered through paper, and to the clear filtrate there are added 50 grams of fullers' earth per liter. The particular variety of fullers' earth is that obtained from Surrey, England, and is imported by Eimer & Amend. The mixture is well shaken at intervals for one-half hour, and the solid is filtered off with the aid of a large Buchner funnel. It is washed with water and finally with alcohol and ether to facilitate subsequent drying.

The extraction of the vitamine from its combination with fullers' earth has been found to be most conveniently accomplished by means of saturated aqueous barium hydroxide solution, used in the proportion of 1 liter per 100 grams of the "activated" fullers' earth. The mixture is violently shaken for three minutes and the solid removed as quickly as possible. A De Laval cream separator, with the disks removed from the bowl, has been found to be very efficient for this purpose. The nearly clear liquid is immediately acidified with a slight excess of concentrated sulphuric acid, added to the actively stirred liquid. About 10 grams, or a moderate excess, of powdered barium carbonate is then added to remove the excess of sulphuric acid. The mixture is filtered after about one-half hour. Nearly saturated lead acetate solution is added to the filtrate until no further precipitate is obtained. The latter is then removed and the excess of lead in the liquid precipitated with hydrogen sulphide. The filtrate from the lead sulphide is then evaporated rapidly under diminished pressure to about one-tenth to one-twentieth its original volume. A white, amorphous precipitate begins to separate when the volume becomes small. This undoubtedly nonvitamine material is filtered off, and the evaporation is continued in a vacuum desiccator. Additional amounts of the amorphous white product separate and are removed from time to time. The liquid will finally be reduced to a thick, viscous mass, which yields no further quantities of the amorphous white precipitate. This crude vitamine extract may then be evaporated to complete dryness in a vacuum desiccator, and in this condition it appears to retain its antineuritic properties indefinitely. One such sample, after having been kept in the laboratory for almost two years, was found to be very active when tested on pigeons.

The test for antineuritic properties, which has been used exclusively upon the vitamine fractions obtained in this work, is con-

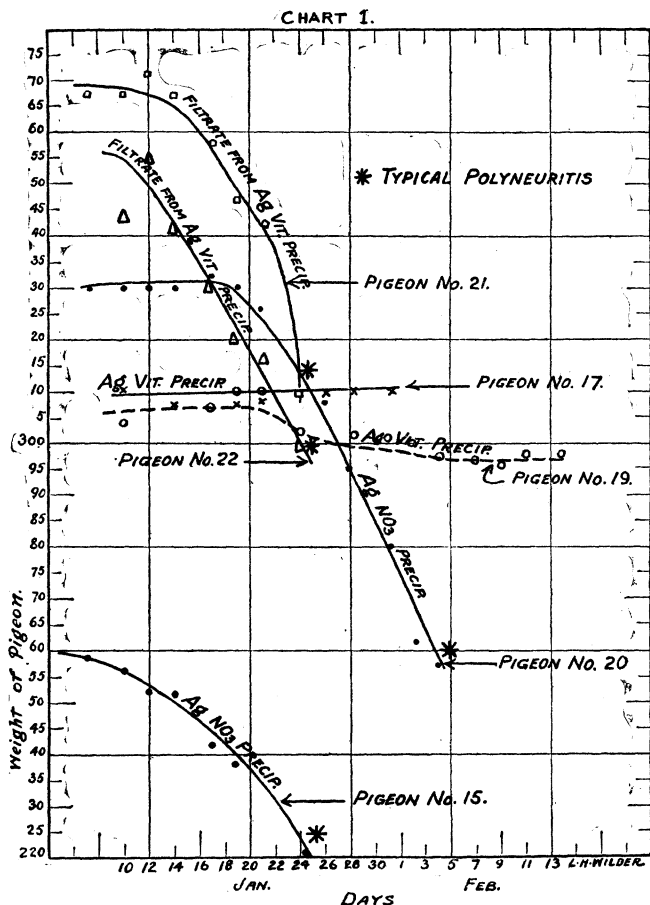
ducted as follows: Groups of about 10 pigeons each are kept in inclosures of some 400 cubic feet and supplied with water and ample amounts of polished rice. Each pigeon is numbered by means of a leg band and is weighed three times weekly. The samples to be tested are administered in gelatin capsules immediately after weighing each pigeon. Maintenance of weight under these conditions shows that the sample being tested contains at least the minimum amount of vitamine required to replace the deficiency of the rice diet. Rapid loss in weight, followed by polyneuritis, occurs among the control pigeons and those receiving doses of samples deficient in antineuritic vitamine.

Using an amount of the crude vitamine extract, prepared as described above, from 300 grams of "activated" fullers' earth, it has been found that after diluting to a volume of about 25 cubic centimeters and gradually adding an almost saturated aqueous silver nitrate solution, a voluminous silver precipitate is obtained. This is removed most conveniently by centrifugation and is washed once by centrifugation, using an amount of water equal to the volume of the precipitate. The decanted solution and wash water, after being tested with the reagent for complete precipitation, are mixed and filtered. To this solution is then added an excess of fairly concentrated aqueous ammoniacal silver nitrate solution, made by adding ammonia to aqueous silver nitrate until the black precipitate, which at first separates, just redissolves. This causes the separation of another voluminous silver precipitate, which is likewise removed by centrifugation and washed as before. These two precipitates and the filtrate from the second one were subjected to tests on pigeons and it was found that of the three samples, the second, obtained by means of ammoniacal silver nitrate, was highly antineuritic.

The results of this experiment are shown in Chart 1. For convenience, the precipitate obtained by means of ammoniacal silver nitrate is designated as silver vitamine precipitate, "Ag. Vit. Precip." The results show very strikingly that neither the silver nitrate precipitate nor the filtrate from the ammoniacal silver nitrate precipitate contains an appreciable amount of the protective vitamine.

When the ammoniacal silver nitrate precipitate, obtained as above described, is suspended in water, and a slight excess of hydrochloric acid is added, the silver is rapidly transformed to silver chloride, which may be easily removed from the clear aqueous solution. The latter, when subjected to slow evaporation in a vacuum desiccator containing stick sodium hydroxide as the drying agent, begins to yield well-formed crystals when the volume is reduced to about 10 cubic centimeters. Several crops of these crystals were easily obtained, but, when tested on pigeons, were found to possess no antineuritic properties. (See Chart 2.) When the mother liquor had been

reduced to a volume of about 3 cubic centimeters, it began to change in color from pale yellowish to reddish and showed no further tendency to yield crystals. In one instance, when the evaporation was accidentally carried further, the whole mass turned to a black, thick, viscous liquid. If the slightly reddish liquid is diluted with a small volume of water and aqueous silver nitrate solution added carefully, silver chloride continues to separate until the excess of hydrochloride has just been removed. The end point for this removal is



very sharp. The filtrate from this silver chloride precipitate, when treated with ammoniacal silver nitrate, yields again the voluminous silver vitamine precipitate, which now, however, is free from the crystallizable inactive fraction described above. The yield of dried precipitate from 300 grams of "activated" fullers' earth was 0.7 gram. Results of the tests of the above-mentioned crystals, as well as of the ammoniacal silver nitrate precipitate as originally obtained, "Crude Ag. Vit. Precip.," and after removal of the inactive

readily yields up its antineuritic constituent when suspended in dilute hydrochloric acid. It appears to suffer no change on drying, and the present experiments show that samples still protect pigeons on a rice diet from polyneuritis after a period of nearly three weeks.¹

A determination of the silver present, made by ignition, showed 54.85 per cent Ag. The doses given the pigeons shown on Chart 2 were 0.004 gram on alternate days, i. e., 0.002 gram per day of the silver compound, which is equivalent to slightly less than 0.001 gram of the antineuritic portion of the material.

Whether the silver vitamine precipitate obtained as described above is a pure compound is, of course, not known at present. It is possible that two or more substances are present in combination with the silver and that different samples which are prepared will vary somewhat in composition. It is believed, however, to be an exceptionally favorable product on which to concentrate efforts toward the identification of the antineuritic vitamine. Attention is, therefore, now being directed toward this part of the problem.

INDEX TO PUBLIC HEALTH REPORTS, VOL. 35, PART 2, 1920.

The index, with title page, to Vol. 35, Part 2 of Public Health Reports for 1920 is now available and may be had on application to the Surgeon General, United States Public Health Service, Washington, D. C.

DEATHS DURING WEEK ENDED MAR. 19, 1921.

Summary of information received by telegraph from industrial insurance companies for week ended Mar. 19, 1921, and corresponding week, 1920. (From the "Weekly Health Index," Mar. 22, 1921, issued by the Bureau of the Census, Department of Commerce.)

	Week ended Mar. 19, 1921.	Corresponding week, 1920.
Policies in force.....	46, 298, 930	41, 997, 632
Number of death claims.....	9, 434	13, 276
Death claims per 1,000 policies in force.....	10.6	16.5

¹ NOTE.—Since this was written the tests (Chart 2) have been continued for an additional period of three weeks, during which time no polyneuritic symptoms developed in any case. Each bird, however, decreased slightly in weight, possibly as a result of the absence of a growth-promoting principle in the purified antineuritic product.